

Flower-feeding affects mating performance in male oriental fruit flies *Bactrocera dorsalis*

TODD E. SHELLEY USDA-APHIS and Hawaiian Evolutionary Biology Program, University of Hawaii, U.S.A.

Abstract. 1. Males of the oriental fruit fly *Bactrocera dorsalis* are attracted to and feed on flowers of the golden shower blossom *Cassia fistula*. Flowers of this plant contain methyl eugenol, the metabolites of which apparently function in the synthesis of male sex pheromone.

2. The goal of the study reported here was to determine whether feeding on *C. fistula* flowers enhanced male mating success. Mating frequencies of unfed (control) and fed (treated) males were compared in trials conducted 0 (same day), 2, 7, or 21 days after treated males were exposed to the flowers. Trials were performed using flowers from three trees of *C. fistula* to investigate whether the effects of floral feeding were similar among different plants.

3. For all three trees, treated males accounted for a disproportionately large number of matings in trials performed 0, 2, and 7 days after floral feeding by the treated males. For two of the trees, treated males also had a mating advantage 21 days after flower-feeding.

4. Additional tests were conducted to compare female attraction to perch sites of control and treated males. When at a lek, males exhibit rigorous wing-fanning behaviour, presumably to increase dispersal of the sex pheromone. Floral feeding had no significant effect on the level of wing-fanning. Significantly more female sightings were recorded for perches of treated than control males, however, suggesting that the treated males produced a pheromone more attractive to females than did control males.

Key words. *Bactrocera dorsalis*, flower-feeding, mating success, pheromone, Tephritidae.

Introduction

Males of various tephritid fruit fly species are attracted to particular chemical compounds variously referred to as parapheromones, lures, or male attractants (Sivinski & Calkins, 1986; Jang & Light, 1996). In some instances, the lure is synthetic and presumed to chemically mimic a natural (but as yet unidentified) substance. For example, males of the Mediterranean fruit fly *Ceratitidis capitata* are attracted to the synthetic compound trimedlure, which is frequently employed in surveys for this agricultural pest (Cunningham, 1989). In other cases, however, the lure is known to be a naturally occurring, plant-borne compound. In the large tropical genus *Bactrocera*, for example, males of many species are attracted to methyl eugenol, a compound found in leaves, flowers, or

fruits of at least 10 plant families (Fletcher, 1987; Metcalf, 1990). In the case of the oriental fruit fly *Bactrocera dorsalis*, male attraction is so powerful that methyl eugenol, when mixed with an insecticide, has eradicated this species from entire islands in the western Pacific (Steiner *et al.*, 1965, 1970).

Despite the frequent use of lures in control programmes, little attention has been given to the biological function underlying male attraction to these chemicals. In the case of *B. dorsalis*, recent data of Nishida *et al.* suggest that methyl eugenol has an important role in sexual communication and mating competition. Nishida *et al.* (1988) demonstrated that males fed pure methyl eugenol concentrated metabolites of this compound in their rectal glands (the presumed site of sex pheromone production) whereas methyl eugenol-deprived males did not. Tan and Nishida (1996) confirmed this result and also noted (as had Shelly & Dewire, 1994) that methyl eugenol-fed males had a mating advantage over control (unfed) males. Importantly, these same results – sequestration of metabolic derivatives and enhanced mating success – were

Correspondence: Dr T. E. Shelly, Hawaiian Evolutionary Biology Program, 3050 Maile Way, University of Hawaii, Honolulu, HI 96822, U.S.A. E-mail: tshelly@hawaii.edu

noted when *B. dorsalis* males were provided with flowers of *Fagraea berteriana*, a natural source of several methyl eugenol-like compounds (Nishida *et al.*, 1997).

While this latter finding suggests a link between methyl eugenol ingestion and sexual selection in *B. dorsalis*, investigations of additional methyl eugenol-containing plants are needed to assess more rigorously the strength and generality of this association. The chief goal of the study reported here was therefore to examine the effects of feeding on the mating success of oriental fruit flies for another methyl eugenol-containing plant, the legume *Cassia fistula* (Kawano *et al.*, 1968). In addition, two related, but previously untested, hypotheses were investigated. First, mating trials were conducted using several individuals of *C. fistula* to test whether effects on male mating frequency were uniform among different plants. Second, as described below, male oriental fruit flies produce a sex pheromone attractive to females, and female arrivals to fed and unfed males were compared to determine whether plant feeding affected male attractiveness via possible changes in the quantity or quality of the pheromonal signals produced.

Methods

Mating behaviour of B. dorsalis

The following summary derives from observations made of wild flies in the laboratory (Fletcher, 1987) or in the field (Shelly & Kaneshiro, 1991). *Bactrocera dorsalis* appears to display a lek mating system. Males aggregate on the foliage of host trees approximately 1 h before dusk and defend individual leaves as mating territories. While perching, males engage in vigorous wing-fanning (also termed *pheromone-calling*), an activity that both produces an audible buzz and disperses a pheromone attractive to females. Upon detecting a female in their territory, males immediately cease wing-fanning and jump on the female. The female then either decamps or copulation ensues. Mating couples remain paired throughout the night and separate at sunrise.

Mating frequency

Flies used in the mating trials were from a laboratory stock started with 200–300 adults reared from field-collected mangoes. The colony was housed in a large screen cage with superabundant food (a mixture of honey and protein hydrolysate) and water. Ripe papayas were provided periodically for oviposition. Room temperature was maintained at 20–22 °C and 65–75% RH; under these conditions, generation time was about 4 weeks. The experiments were performed when the colony was 6–7 months old, and correspondingly the flies used were six to seven generations removed from the wild. Adults used in the trials were separated within 7 days of eclosion, well before reaching sexual maturity at 15–20 days of age (Foote & Carey, 1987).

Mating trials were performed in the same manner for all experiments. Three treated males given prior exposure to flowers of *C. fistula* (as described below) and three control males having no exposure to flowers were placed with three females into screen cages 2–3 h before sunset. The males were marked 1 day before testing by cooling them for several minutes and placing a dot of enamel paint on the thorax. This procedure had no adverse effects, and males resumed normal activities within minutes of handling. The cages, 30 cm cubes with one side fitted with a cloth sleeve, were kept adjacent to a large, west-facing window, and room lights were extinguished at least 2 h before sunset. Identities of mating males were recorded 3–4 h after sunset under dim light. Ten to 14 cages were observed on a given day. All flies were used for a single trial only.

The following procedure was used to expose males to *C. fistula* flowers in all experiments (except one as described below). Flowering stems were removed from the tree, put in water, transported to the laboratory, and placed into screen cages (30 cm cubes) at 08.00 hours (cages used for floral exposure were not used in the mating trials). Floral counts were made to standardise availability, and 140–160 flowers were placed in the cages (*C. fistula* trees bloom for several weeks and contain thousands of flowers at any one time). Forty males were then introduced to the cages (along with food and water) and left until 15.00 hours. Flower-containing cages were isolated to prevent inadvertent exposure of floral volatiles to control males. Although systematic observations were not made, most males moved onto the petals and began touching them with their mouthparts within minutes of being placed in the cages. Feeding was observed throughout the day, although the number of flower-perching males declined markedly by the afternoon.

The mating trials were designed to test three main hypotheses. (1) The effect of treatment (presence or absence of floral exposure) on male mating success was examined for various post-exposure intervals. Mating trials were conducted 0 (same day exposure), 2, 7, or 21 days following floral exposure to the treated males. In a given trial, the control and treated males were approximately the same age; treated males were given flowers when 18–21 days old (females in all trials were 20–25 days of age). (2) The effect of tree identity on the mating success of treated males was investigated. Mating trials were performed using flowers from single *C. fistula* growing in three different areas on the island of Oahu, Hawaii (21.30°N, 158°W). Two of the sites – Manoa and Makiki – were adjacent sections of Honolulu with similar temperature and rainfall regimes; the third (Waimanalo) was a much wetter area approximately 30 km NW of Honolulu. Over the course of the study, males were seen feeding on flowers of the Makiki (three sightings) and Waimanalo (≈ 25 sightings) trees. (3) To determine whether exposure to floral volatiles alone would influence mating success, an experiment was performed in which treated males were given flowers covered with screen mesh that prevented direct contact. The flowers used in this experiment were from the Manoa tree, and a post-exposure interval of 2 days alone was used.

Mate attraction

Because males from the laboratory stock were reluctant to wing-fan in the mini-cages (see below), the flies used in this experiment were from a colony maintained by the Tropical Fruit, Vegetable, and Ornamental Crop Laboratory (USDA-ARS), Honolulu, for ≈ 90 generations. Pupae were obtained 2 days before eclosion, and adults were separated by sex within 5 days of emergence (sexual maturity in this stock is attained at ≈ 10 days of age; Foote & Carey, 1987).

Tests of male attractiveness were conducted on groups of eight males (four treated, four control) in a large screen cage ($1.2 \times 0.6 \times 0.6$ m) that contained three potted plants (*Ficus* sp.). Treated males were exposed to flowers in the manner described above (all flowers were taken from the Manoa tree) when 14–16 days old and were used 2 or 7 days after exposure. In a given trial, control and treated males were the same age. Approximately 3 h before sunset, males were placed singly in mini-cages, which, in turn, were placed at specific locations on the plants. The cylindrical mini-cages (6 cm long \times 3 cm diameter) were made of wire screening and were suspended from branches with a wire hook. The mean nearest-neighbour distance between mini-cages was 20 cm (range 16–22 cm). Mini-cages were placed at the same locations for each trial, but the type of male (control or treated) at a given location was assigned randomly at the start of a trial. Immediately after the males were in place, 40 females (14–16 days old) were released into the cage. The room lights were extinguished, and the large cage, which was near a west-facing window, received only natural light.

Starting 1.5 h before sunset, males were observed continuously for the start of wing-fanning by any male. At its onset, males were checked at 1-min intervals for the presence/absence of wing-fanning and for the number of females resting on the individual mini-cages. Wing-fanning level was considered to be a direct indicator of pheromone production, a legitimate assumption because pheromone emission occurs only during wing-fanning (Ohinata *et al.*, 1982). Data on female visitation reflected numbers of female sightings alone; because females were not marked, neither the number of different females arriving nor the duration of individual visits was known. Observations were made over 4 days for both 2- and 7-day post-exposure intervals ($n = 16$ males for both control and treated groups) for periods of 30–36 min (intervals between first wing-fanning and sunset).

Statistical analyses

The mating frequency of control and treated males was compared for a particular post-exposure interval by combining data over all cages over all days and performing a binomial test (using the normal approximation and Z scores) to detect deviation from random mating (i.e. 50% of matings achieved by each male type). Between-tree comparisons were made separately for the different post-exposure intervals using the log-likelihood ratio (or G test, where $2 \times G$ approximates χ^2). Initial tests included all three trees; if significant variation was

detected, additional pairwise comparisons were drawn. In mate attraction tests, the proportion of the total observation time spent calling and number of female sightings per 10 min of observation time were computed for individual males, and these values were used in Mann–Whitney U-tests comparing control vs. treated males. Simple linear regressions of female sightings vs. male calling were calculated for control and treated males, respectively, and slopes were compared via a *t*-test. All statistical procedures follow Zar (1996).

Results

Mating frequency

For trials with the Manoa and Waimanalo trees, treated males accounted for a disproportionately large number of matings in all post-exposure intervals (Fig. 1). For the Manoa tree, treated males achieved 89% of all matings in trials conducted on the same day as exposure and 61–66% in trials conducted 2–21 days after exposure. For the Waimanalo tree, treated males obtained 65–69% of the matings over all post-exposure intervals. For the Makiki tree, treated males had a mating advantage in trials conducted within 7 days of exposure (accounting for 66–69% of all matings) but not in trials conducted 21 days after exposure (accounting for only 48% of all matings).

As these findings suggest, flowers from the different trees had similar effects on male mating success (Fig. 1). For post-exposure intervals of 2 and 7 days, the relative success of treated males was similar across all three trees ($\chi^2 = 0.8$ and 0.6, respectively, $P > 0.05$ in both cases). For trials conducted

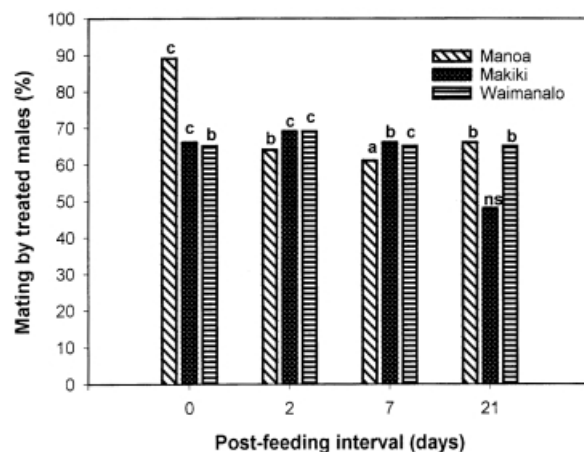


Fig. 1. Proportion of matings by treated males in trials performed with post-exposure intervals of 0, 2, 7, or 21 days using flowers from *C. fistula* trees growing in three different locations. Sample sizes (total matings) ranged from 94 to 125. Letters above bars correspond to significance levels for binomial tests of random mating, where *a*, *b*, and *c* indicate $P < 0.05$, 0.01, 0.001, respectively, and *ns* indicates $P > 0.05$.

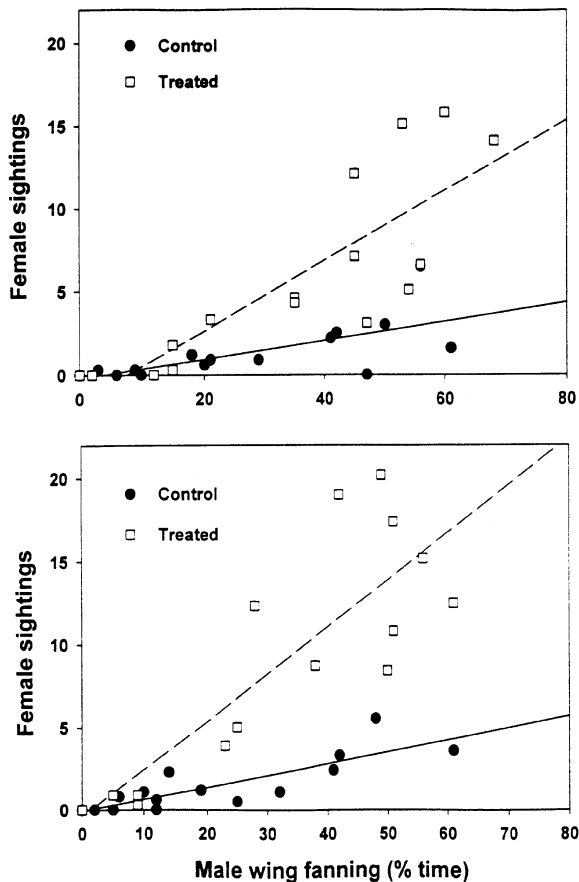


Fig. 2. Relationship between male wing-fanning activity and female sightings (number per 10 min) for control and treated males in trials conducted 2 (top) or 7 (bottom) days after treated males fed on *C. fistula* flowers. Least-squared regressions: 2-day – control: $Y = 0.06X - 0.3$, $r^2 = 0.53$; treated: $Y = 0.21X - 1.6$, $r^2 = 0.68$. 7-day – control: $Y = 0.07X - 0.04$, $r^2 = 0.69$; treated: $Y = 0.29X - 0.52$, $r^2 = 0.74$.

on the day of exposure, there was significant variation among trees in the relative success of treated males ($\chi^2 = 16.6$, $P < 0.001$), reflecting the much higher mating success observed for males exposed to the Manoa tree than the Makiki ($\chi^2 = 13.8$) or the Waimanalo ($\chi^2 = 13.0$) trees ($P < 0.001$ in both cases; no significant difference was found between the latter two trees). Significant variation was also found for trials conducted 21 days after exposure ($\chi^2 = 7.6$, $P < 0.05$), reflecting the lower mating success observed for males exposed to the Makiki tree than the Manoa ($\chi^2 = 4.1$) or the Waimanalo ($\chi^2 = 5.2$) trees ($P < 0.05$ in both cases; no significant difference was found between the latter two trees).

Exposure to volatiles alone did not enhance male mating success. Males that were given screen-covered (and hence inaccessible) flowers obtained 56% (76/136) of all matings, a proportion not significantly different from that expected by chance ($Z = 1.4$, $P > 0.05$).

Mate attraction

Floral feeding had no detectable effect on male wing-fanning levels (Fig. 2). On average, treated males tested 2 days after floral exposure were fanning for 35% of the observation period compared to 27% for the control males ($U = 159.5$, $P > 0.05$). In the trials with a 7-day post-feeding interval, treated and control males were wing-fanning for an average of 31 and 23% of the observation period, respectively ($U = 154$, $P > 0.05$). Although wing-fanning levels were similar, there were significantly more female sightings for treated than for control males for both 2- and 7-day post-feeding intervals (Fig. 2). Treated males tested 2 days after floral feeding had an average of 5.8 female sightings per 10 min compared to only 1.3 for control males ($U = 195.5$, $P < 0.05$). Similarly, treated males tested 7 days after floral feeding averaged 8.3 female sightings per 10 min compared to 1.4 for control males ($U = 200$, $P < 0.01$). Consistent with these findings, slopes of female sightings vs. male calling were greater for treated than for control males in both tests (2-day: $t = 3.5$, $P < 0.01$; 7-day: $t = 5.8$, $P < 0.001$; Fig. 2). Slopes were similar between control ($t = 0.50$) and treated ($t = 1.33$) males in the 2- and 7-day trials ($P > 0.05$ in both cases).

Discussion

The study reported here provides evidence, for a second plant family, that feeding on a natural source of methyl eugenol increases the mating success of male oriental fruit flies. This advantage was evident 7 days after floral feeding on all three *C. fistula* trees tested, and as long as 21 days after feeding on two of the trees. Based on the mate attraction experiments, enhanced mating success may have reflected the production of a more attractive signal by flower-fed males. Pheromonal signals of *B. dorsalis* fed pure methyl eugenol have been shown to be more attractive to females than those of control males (Shelly & Dewire, 1994; see also Hee & Tan, 1998 for data on *B. papayae*), but the present study represents the first demonstration of increased signal attractiveness via feeding on a natural source of male lure for any tephritid species. As shown for methyl eugenol-fed males, flower-fed males presumably ingested the lure and incorporated methyl eugenol derivatives into their sex pheromone. Ingestion of pheromonal precursors from natural sources has been demonstrated for adult males of various Lepidoptera (e.g. Krasnoff & Dussourd, 1989) and Coleoptera (e.g. Byers, 1982).

While females mated preferentially with floral-fed males, the adaptive basis of this preference remains unknown. Mating with methyl eugenol-fed males does not appear to increase female fecundity: egg production during an 8-week period immediately following mating did not differ significantly between females mated to treated (methyl eugenol-fed) males or control (methyl eugenol-deprived) males (T. E. Shelly, unpublished). Likewise, whether or not a male fed previously on methyl eugenol had no significant effect on the proportion of eggs hatching or post-mating survival of females (T. E. Shelly, unpublished). In these experiments, males were fed

pure methyl eugenol, and it is possible that the combination of methyl eugenol and some other co-occurring compounds may act to increase female fecundity. Experiments using natural sources of methyl eugenol are required to evaluate this possibility.

Alternatively, the presence of methyl eugenol-derived pheromonal components in the male signal may indicate a superior ability to locate natural sources of methyl eugenol in the wild. As such, by selecting males whose pheromones contain methyl eugenol metabolites, females may increase the odds that their sons will have high ability to locate methyl eugenol sources and hence enjoy high mating success. This scenario depicts a case of runaway selection, whereby female choice provides indirect benefits via a trait that confers an advantage to her sons in sexual competition but is *arbitrary* with respect to offspring viability (Andersson, 1994). This explanation still begs the question as to the origin of the female preference. One interesting possibility is that females prefer male pheromone containing metabolites of methyl eugenol because it triggers a strong, pre-existing sensory bias that evolved in a different context (e.g. food searching). Pheromones of other male tephritids have, in fact, been shown to contain certain compounds that mimic food and host odours (Baker *et al.*, 1990; Robacker & Warfield, 1993). Thus, methyl eugenol-bearing pheromone may represent a *sensory trap* (West-Eberhard, 1984) or a case of *sensory exploitation* (Ryan, 1990).

A related possibility, also based on the notion of a *sensory trap*, is that the male sex pheromone in *B. dorsalis* serves to advertise the location of resources critical to females (Landolt, 1997). In this case, male pheromones with methyl eugenol-derived metabolites are more attractive to females because they provide a stronger signal for the location, not of particular males *per se*, but of food or ovipositional resources. By advertising a resource more successfully, males that had consumed methyl eugenol might therefore gain increased contacts with potential mates. While possible for other species, it seems unlikely that the resource advertisement hypothesis applies to *B. dorsalis* because males signal for a very short period each day (only about 1 h) and females at a natural lek perched exclusively on leaves (and not fruits, i.e. potential oviposition sites) and were not seen feeding.

In conclusion, several factors, the strong attraction of males to methyl eugenol, the pronounced mating advantage of methyl eugenol-fed males, and the long duration of this advantage, indicate collectively that methyl eugenol-containing plants have an important influence on the mating system of *B. dorsalis*. At present, this influence appears to derive mainly from the effect of methyl eugenol on male attractiveness (and thus female mate choice) and not from any spacing effect of methyl eugenol-containing plants on mate-searching strategies. Based on casual observations of the golden shower trees, sexual activities do not occur on methyl eugenol-containing plants. At the Waimanalo tree, males appeared to be more abundant in the morning than in the afternoon, and no males were seen during a single check made 30 min before sunset. Moreover, the only lek observed in the field did not occur near any obvious source of methyl eugenol (Shelly & Kaneshiro,

1991). These observations, coupled with the strong flight ability of *B. dorsalis*, suggest that leks and methyl eugenol-bearing plants are distributed independently of one another.

Acknowledgements

I thank Stacey Fong, Emma and Miranda Shelly, and Meredith Whitney for help in maintaining the flies and collecting data, Rick Kurashima for supplying flies, and Pete Landolt and Tom Phillips for comments on an earlier draft. This work was supported by funds from USDA-CSRS (9401860).

References

- Andersson, M. (1994) *Sexual Selection*. Princeton University Press, Princeton, New Jersey.
- Baker, P.S., Howse, P.E., Ondarza, R.N. & Reyes, J. (1990) Field trials of synthetic sex pheromone components of the male Mediterranean fruit fly (Diptera: Tephritidae) in southern Mexico. *Journal of Economic Entomology*, **83**, 2235–2245.
- Byers, J.A. (1982) Male specific conversion of the host plant compound myrcene to the pheromone (+)-ipsdienol in the bark beetle *Dendroctonus brevicornis*. *Journal of Chemical Ecology*, **8**, 363–372.
- Cunningham, R.T. (1989) Population detection. *World Crop Pests, Vol. 3B, Fruit Flies, their Biology, Natural Enemies and Control* (ed. by A. S. Robinson and G. Hooper), pp. 169–173. Elsevier, Amsterdam.
- Fletcher, B.S. (1987) The biology of dacine fruit flies. *Annual Review of Entomology*, **32**, 115–144.
- Foot, D.H. & Carey, J.R. (1987) Comparative demography of a laboratory and a wild strain of the oriental fruit fly, *Dacus dorsalis*. *Entomologia experimentalis et applicata*, **44**, 263–268.
- Hee, A.K.W. & Tan, K.H. (1998) Attraction of female and male *Bactrocera papayae* to conspecific males fed with methyl eugenol and attraction of females to male sex pheromone components. *Journal of Chemical Ecology*, **24**, 753–764.
- Jang, E.B. & Light, D.M. (1996) Olfactory semiochemicals of tephritids. *Fruit Fly Pests* (ed. by B. A. McPherson and G. J. Steck), pp. 73–90. St Lucie Press, Delray Beach, Florida.
- Kawano, Y., Mitchell, W.C. & Matsumoto, H. (1968) Identification of the male oriental fruit fly attractant in the golden shower blossom. *Journal of Economic Entomology*, **61**, 986–988.
- Krasnoff, S.B. & Dussourd, D.E. (1989) Dihydropyrrolizine attractants for arctiid moths that visit plants containing pyrrolizidine alkaloids. *Journal of Chemical Ecology*, **15**, 47–60.
- Landolt, P.J. (1997) Sex attractant and aggregation pheromones of male phytophagous insects. *American Entomologist*, **43**, 12–22.
- Metcalf, R.L. (1990) Chemical ecology of Dacinae fruit flies (Diptera: Tephritidae). *Annals of the Entomological Society of America*, **83**, 1017–1030.
- Nishida, R., Tan, K.H., Serit, M., Lajis, N.H., Sukari, A.M., Takahashi, S. *et al.* (1988) Accumulation of phenylpropanoids in the rectal glands of males of the oriental fruit fly, *Dacus dorsalis*. *Experientia*, **44**, 534–536.
- Nishida, R., Shelly, T.E. & Kaneshiro, K.Y. (1997) Acquisition of female-attracting fragrance by males of the oriental fruit fly from a Hawaiian lei flower, *Fagraea berteriana*. *Journal of Chemical Ecology*, **23**, 2275–2285.
- Ohinata, K., Jacobson, M., Kobayashi, R.M., Chambers, D.L.,

- Fujimoto, M.S. & Higa, H.H. (1982) Oriental fruit fly and melon fly: biological and chemical studies of smoke produced by males. *Journal of Environmental Science and Health, A*, **17**, 197–216.
- Robacker, D.C. & Warfield, W.C. (1993) Attraction of both sexes of Mexican fruit fly, *Anastrepha ludens*, to a mixture of ammonia, methylamine, and putrescine. *Journal of Chemical Ecology*, **19**, 2999–3016.
- Ryan, M.J. (1990) Sexual selection, sensory systems and sensory exploitation. *Oxford Surveys in Evolutionary Biology*, **7**, 157–195.
- Shelly, T.E. & Kaneshiro, K.Y. (1991) Lek behavior of the oriental fruit fly in Hawaii. *Journal of Insect Behavior*, **4**, 235–241.
- Shelly, T.E. & Dewire, A.M. (1994) Chemically mediated mating success in male oriental fruit flies, *Bactrocera dorsalis* (Diptera: Tephritidae). *Annals of the Entomological Society of America*, **87**, 375–382.
- Sivinski, J. & Calkins, C.O. (1986) Pheromones and parapheromones in the control of tephritids. *Florida Entomologist*, **69**, 157–168.
- Steiner, L.F., Hart, W.G., Harris, E.J., Cunningham, R.T., Ohinata, K. & Kamakahi, D.C. (1970) Eradication of the oriental fruit fly from the Mariana Islands by the methods of male annihilation and sterile insect release. *Journal of Economic Entomology*, **63**, 131–135.
- Steiner, L.F., Mitchell, W.C., Harris, E.J., Kozuma, T.T. & Fujimoto, M.S. (1965) Oriental fruit fly eradication by male annihilation. *Journal of Economic Entomology*, **58**, 961–964.
- Tan, K.H. & Nishida, R. (1996) Sex pheromone and mating competition after methyl eugenol consumption in the *Bactrocera dorsalis* complex. *Fruit Fly Pests* (ed. by B. A. McPherson and G. J. Steck), pp. 147–153. St Lucie Press, Delray Beach, Florida.
- West-Eberhard, M.J. (1984) Sexual selection, competitive communication and species-specific signals in insects. *Insect Communication* (ed. by T. Lewis), pp. 283–324. Academic Press, New York.
- Zar, J.H. (1996) *Biostatistical Analysis*. Prentice Hall, London.

Accepted 22 May 1999